The Institute of Biology's
Studies in Biology no. 40

Endogenous Plant Growth Substances

Second Edition

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General Preface to the Series

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Preface

The study of the control of growth and differentiation in living organisms is one of the major preoccupations of current biological research, and this book looks at just one area of the subject. Many aspects of plant growth and development are controlled through the mediation of endogenous plant growth substances or plant hormones, and research on these subjects has a fairly long history. The discovery of the many types of synthetic chemical with which we can control growth artificially in agriculture and horticulture is one result of such studies, though these substances fall largely outside the scope of this book in which I have tried to outline the present state of our knowledge of the plant hormones and to show some of the ways in which the many fascinating problems of their study have been approached. In this edition I have tried to include some of the more important advances which have taken place since the book first appeared and to reorganize some parts of the material as well.

I am grateful to all who have helped, knowingly or unknowingly, in the preparation of the book. The gestation period of these pages included some months which I spent working in the laboratory of the great French plant physiologist J. P. Nitsch. The book is dedicated in respect and affection to his memory, and also to all his colleagues in gratitude for their stimulating and friendly hospitality.

Wye, 1979

T. A. H.

Contents

1 Introduction

Plants normally grow and develop in an orderly, organized way. This is one of the many characteristics they share with all living organisms. Such orderly growth and development may be affected in many ways by the environment, which in some cases plays a very important part in controlling or triggering off various patterns of development. For example, some plants only produce flowers if the daily light period exceeds a certain critical length, and some will only do so if they have been exposed to cold at an earlier stage in their development. In a general way, however, it is obvious that the essential features of growth and development are built into the genetic constitution of the plant and are thus controlled from within.

The internal control of growth is achieved in many and complex ways and there is a tendency for each plant physiologist to look at such mechanisms from the point of view of his own area of specialization. This can be dangerous because the one thing which is certain is the very highly integrated nature of the controlling processes. None the less, there is no harm in concentrating attention on one aspect of the subject at a time if it is remembered that this is what one is doing. This is the philosophy which provides the justification for this book.

One of the important growth-controlling systems in plants is provided by the so-called 'plant growth substances' or 'plant hormones'. The term 'hormone' is, of course, used by animal physiologists too, and indeed the concept of such chemical messengers in the body was first developed by them in the early part of this century. Animal hormones differ from plant hormones in a number of ways, of which perhaps the most important is that they are produced in specific organs or glands and often have very highly specific effects. Plant hormones, on the other hand, though they may be produced in fairly restricted regions of plants, are manufactured by unspecialized cells and frequently have many different effects upon the plant, depending upon other circumstances.

It will be useful to start with a definition of the terms 'plant growth substance' or 'plant hormone':

> A plant growth substance (or plant hormone) is an organic substance which is produced within a plant and which will at low concentrations promote, inhibit or qualitatively modify growth, usually at a site other than its place of origin. Its effect does not depend upon its calorific value or its content of essential elements.

Not all plant hormones always fit this definition exactly, but it provides a useful working description of the substances we shall be discussing.

An immediate problem which arises is that there are many substances known which, when applied to plants, have effects which closely resemble those of the plant hormones. Some of these are actual chemical analogues of the endogenous plant hormones and some are not, but all can be synthesized in the laboratory. Such chemicals are very important in many types of research (see later), for example, some of the so-called hormone weed-killers fall into this category. Such substances are usually called 'plant growth regulators' or, more simply, 'plant regulators'. In many cases plant regulators of various kinds have been successfully used in the study of processes controlled internally by plant hormones.

One of the major difficulties of the study of endogenous plant growth substances is that the quantities of these chemicals present in plants are always very small indeed by the standards of normal techniques of chemical analysis. This problem is discussed further in Chapter 2. Another problem is that a very great deal of the relevant work is based on what is really circumstantial evidence derived from experiments in which the chemical, or a close relative of it, is applied to a plant from the outside. The reasoning in such cases is essentially something like this:

(*a*) we know that a substance *X*, or one very like it, occurs in a certain plant;
(*b*) we have a supply of substance *Y*, which is very similar to substance *X*;
(*c*) when applied to the relevant plant, substance *Y* causes a specific response (e.g. stem elongation);
(*d*) therefore it is likely, or possible, that substance *X* has a role in controlling stem elongation in this plant.

This is an over-simplification, of course, but there is no doubt that much of our understanding of plant growth substances depends on evidence which is at least partly of this kind. This is perfectly acceptable provided that we constantly bear in mind the possible sources of error in such arguments, and eventually try to confirm the conclusions by more direct methods.

Before going any further it will be useful to define the main groups of substances with which the rest of this book will be concerned. Not every point in the definitions will be clear at this stage, but it is important that they should be presented now and it may be useful to re-read the definitions later on when some of the more detailed information about the substances has been covered (Chapters 3 and 4).

Five groups of substances will be discussed:

(1) AUXINS These are substances chemically related to indole acetic acid (IAA) (Fig. 1–1), which itself appears to be the principal auxin of many plants. They are characterized by their ability to promote growth in certain biological tests involving the use of excised parts of plants freed as

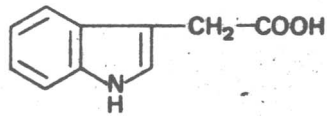

Fig. 1–1 Indole acetic acid (IAA).

far as possible from their own endogenous auxins. Many indole compounds have IAA-like effects, probably because they are converted to IAA by the plant.

Many synthetic growth regulators which are not based on the indole framework have similar effects. A well-known example of one such molecule is 2,4-dichlorophenoxy-acetic acid (2,4-D) which is one of the constituents of many weed-killers (Fig. 1–2).

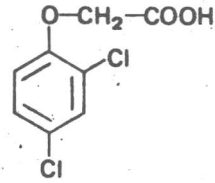

Fig. 1–2 2,4-dichlorophenoxy-acetic acid (2,4-D).

(2) GIBBERELLINS These are substances chemically related to gibberellic acid (usually abbreviated to GA_3), which is a metabolic product of the fungus *Gibberella fujikuroi* and can be obtained from the liquid medium in which the fungus has been cultured. The gibberellin molecule is based on a gibbane skeleton (Fig. 1–3).

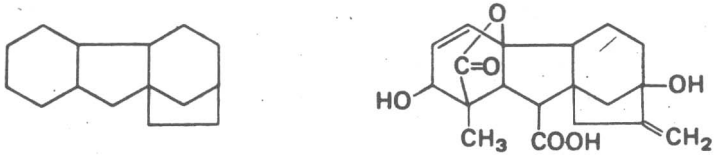

Fig. 1–3 The gibbane carbon skeleton (*left*) which is the basis of the known gibberellins, with the formula of gibberellic acid (gibberellin A_3, GA_3) (*right*).

Many different gibberellins have been found in plants and have been chemically characterized, and to a greater or lesser extent they all share the ability to cause stem elongation when applied to intact plants in the light; certain genetic dwarf strains of maize and peas are particularly sensitive test plants. Many cases exist where substances with properties resembling the known gibberellins have been extracted from plants, but not in quantities permitting exact chemical identification. Such substances are spoken of simply as 'gibberellin-like substances'.

(3) CYTOKININS These are substances which are derivatives of the purine adenine, which is well known as one of the nitrogenous bases in the molecules of the nucleic acids DNA and RNA. They are characterized by

their ability to interact with IAA to promote cell division in cultures of plant cells grown on artificial media, and especially by their property of affecting the patterns of differentiation which occur in such cultures. They have many other properties but this one is critical. An example of a naturally occurring cytokinin is zeatin, which has been obtained from maize grains (Fig. 1–4).

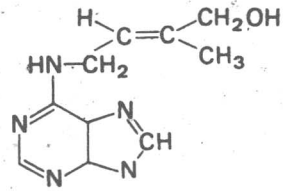

Fig. 1–4 The naturally occurring cytokinin zeatin.

The best-known cytokinin is a substance known as kinetin, but this has not so far been found to occur naturally in plants and should thus be classified as a plant growth regulator by our earlier definition. As with the gibberellins there are many cases of plant extracts containing cytokinin-like substances which are so-called purely on the basis of their properties.

(4) INHIBITORS There are many substances present in plant cells which will, under some conditions, inhibit certain plant processes. Notable amongst these are phenolic compounds (see LEOPOLD and KRIEDMANN, 1975, Chapter 8). However, the substances most able to be considered as inhibitors in the hormonal sense of our definition are those similar in structure and properties to abscisic acid (ABA) (Fig. 1–5). This substance is characterized by its ability to inhibit many growth phenomena in plants, but perhaps especially by its association with bud dormancy in woody plants and with the abscission of leaves in the cotton plant.

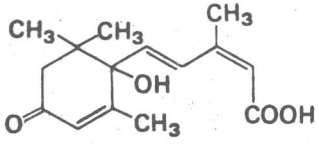

Fig. 1–5 Abscisic acid. This has also been known as Abscisin II and dormin in some of the earlier literature.

(5) ETHYLENE (ethene) (Fig. 1–6) This simple substance has long been known to affect the growth of plants, and since the development of sensitive techniques for detecting it and measuring its concentration, it has become clear that ethylene plays an important part in many growth responses (ABELES, 1973). It seems, for example, to be involved in many auxin-induced growth responses and it plays a part in leaf senescence, abscission and in the ripening of some fruits.

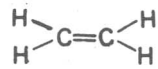

Fig. 1–6 Ethylene.

With the exception of ethylene, it can be seen that we have had to define the groups of plant hormones largely in terms of their properties. Indeed, in most cases they are defined in terms of what they will do to plants when applied exogenously. The reasons for this are largely those of convenience and historical accident. A large part of what we know about the plant hormones has been learned from experiments in which they were applied exogenously to plants (or parts of plants) and it is simpler to define them in terms of these effects than in any other way. This will become clearer as we look at the properties of the hormones in more detail and consider the relation between external application and endogenous role in the control of growth.

The fact that only five groups of plant growth substances are to be discussed does not necessarily imply that there are no other groups of plant hormones to be discovered, a point discussed further in Chapter 8.

A very short sketch of how the plant growth substances came to be discovered will now be useful. Fuller accounts of these studies can be easily found elsewhere, for example ADDICOTT and LYON (1969); PRATT and GOESCHL (1969); WILKINS (1969); PHILLIPS (1971); ABELES (1973); WAREING and PHILLIPS (1978).

1.1 Auxins

Figure 1–7 summarizes some of the crucial stages in the discovery of auxins.

Darwin's classical experiments with grass seedlings exposed to unilateral light showed that phototropic curvature was caused by a response in one part of the organ to a stimulus received elsewhere, and P. Boysen Jensen's work showed that the mediator of the response must be a chemical which moved in the plant. A. Paàl showed that the uneven growth which occurs under unilateral light could be simulated by supplying the chemical stimulus from the apex unilaterally. F. W. Went was able to isolate the chemical messenger by allowing it to diffuse into agar, which was then placed asymmetrically on the decapitated seedling and which caused it to bend. The subsequent discovery of high levels of auxin activity in human urine and the culture filtrates of certain fungi greatly helped the study of the chemical nature of the substance, and indole acetic acid has now been isolated and chemically characterized from a wide variety of plants. Other indole compounds also occur in plants, but it is probable that their auxin-like activity is due to their conversion in the plant to IAA. IAA occurs not only as the free acid in plants, but is also often associated with other molecules, occurring bound to proteins or conjugated with amino acids or sugars (see THIMANN, 1969).

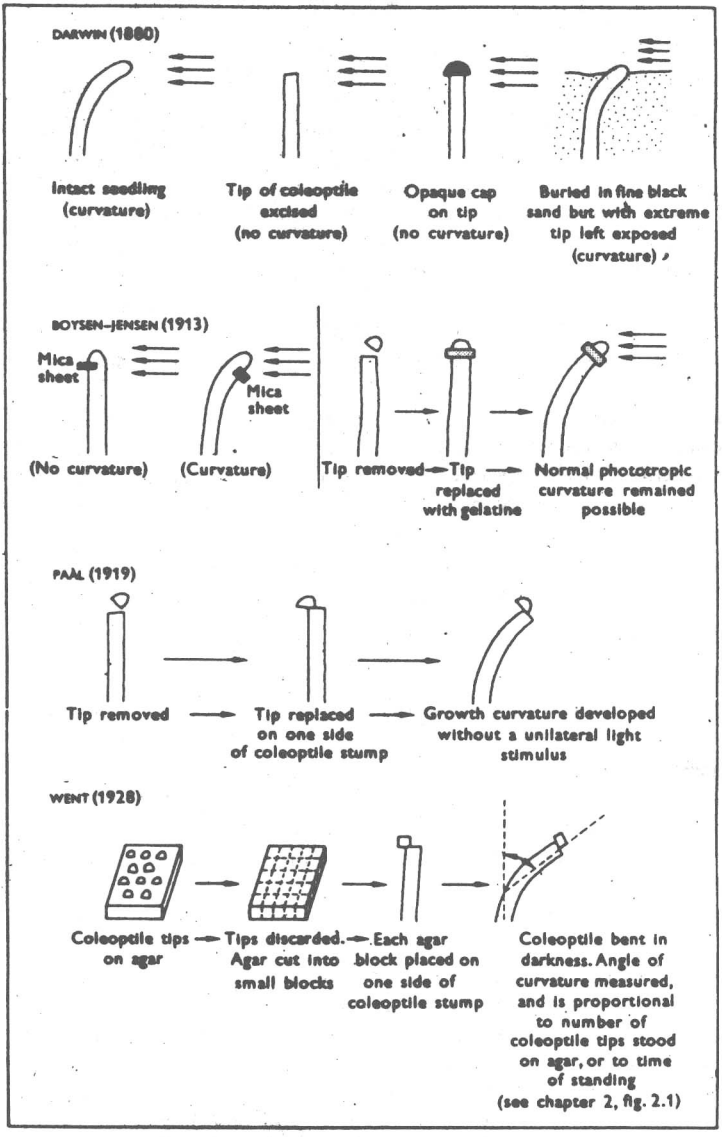

Fig. 1–7 A diagrammatic summary of some important steps in the discovery of auxins. References will be found in WENT and THIMANN (1937). Triple arrows indicate the direction of unilateral light, and all the experiments shown were done using grass seedling coleoptiles. (Modified from WAREING and PHILLIPS, 1978.)

1.2 Gibberellins

The fungus *Gibberella fujikuroi* causes a disease of rice known in Japan as 'bakanae' or 'foolish seedling'. The name derives from the appearance of infected plants which are taller than healthy ones. In 1926 E. Kurosawa showed that this particular symptom could be duplicated by treating plants with the culture filtrate of the fungus. In the following thirty years much effort in Japan was devoted to trying to identify and characterize the causal chemical and to describe the physiological response of plants to it. Much of this early work is in Japanese, but good accounts of it are available in English (e.g. STOWE *et al.*, 1961).

In the early 1950s, workers in the U.S.A. and in England took up the study of gibberellins. As a result of the influx of new workers, the new chemical techniques by then available, and in some cases a certain amount of luck (see, e.g., the paper by STOWE *et al.*, quoted above), the structure of GA_3 was worked out and a vast amount of work on its effects on plants began. The many different physiological effects of GA_3 on plants had led to predictions that a substance similar to it would be found in higher plants, and in fact the first evidence suggesting this was published in 1956 (WEST and PHINNEY, 1956; RADLEY, 1956). Since that time many gibberellins have been isolated, purified, chemically characterized and in some cases their metabolism studied (HEDDEN *et al.*, 1978). Some of these are of fungal origin, some from higher plants and some (e.g. gibberellic acid itself) from both sources.

There are also many reports of substances in plant extracts which are clearly gibberellins but which have not so far been chemically characterized as such. The known gibberellins are referred to by numbers which are approximately in chronological order of their discovery.

1.3 Cytokinins

Studies of plant cells grown under sterile conditions on synthetic nutrient media led to the discovery of this class of plant hormones. F. Skoog and his colleagues at Wisconsin worked with a strain of tobacco in which stem cells would grow in pure culture only if IAA were added. They showed that while cell division would take place in such cultures if vascular tissues were present, isolated pith cells responded only by cell enlargement. Cell division in such pith cultures could be induced by supplying malt extract or coconut milk. It was later shown that autoclaved DNA was highly active and from this work stemmed the isolation and characterization of the powerful cell-division-inducing substance kinetin (K). A good account of this early work is given by FOX (1969).

Kinetin was found to have many effects on plants other than the particular one described and the search for endogenous substances with similar properties in plants became very active. Although many plant extracts were soon found to contain substances with kinetin-like activity it

was not until 1964 that the first naturally occurring cytokinin, called zeatin, was finally characterized by D. S. Letham.

All the cytokinins so far discovered are derivatives of adenine, and since such derivatives are relatively easily synthesized much work has been possible on the relationship between structure and activity.

1.4 Inhibitors

An account of many growth inhibitory substances in plants is given by LEOPOLD and KRIEDMANN (1975) but from the point of view of hormone physiology the history of the most interesting inhibitor (known since 1967 as abscisic acid or ABA) dates from the middle 1960s. The story of ABA is interesting because it is a striking example of several groups of workers reaching the same conclusions by different routes. One group in California, led by H. R. Carns and F. T. Addicott, working on the problem of abscission in the cotton plant, isolated from cotton fruits an inhibitor whose level was associated both with premature abscission in young fruits and with the final abscission of mature ones. They published an account of the structure of this substance in 1965 and called it abscisin II.

At the same time, a group of British researchers led by P. F. Wareing at Aberystwyth, working on the control of dormancy in deciduous trees, isolated an inhibitor which seemed to be associated with this phenomenon. Chemical analysis showed that this inhibitor (which they called dormin) was in fact identical with abscisin II.

A third study, on an inhibitor associated with fruit abscission in lupin pods, culminated in a report by CORNFORTH *et al.* (1966) showing that this too was identical with abscisin II.

Subsequently an enormous amount of attention has been devoted to ABA, and to the study of related inhibitors in plants.

1.5 Ethylene

Early in this century it was shown that traces of ethylene in illuminating gases in the laboratory affected geotropic behaviour of stems and roots and caused stunting and increased radial growth of pea stems. The production of ethylene by some ripening fruits and its effect on triggering off ripening in other fruits were also well established in the first part of the century. As early as 1935 there were suggestions that ethylene might be regarded as a hormone and the production of ethylene by various plants was shown at about the same time. However, until recently the difficulty of detecting and measuring the very minute quantities of ethylene involved in many plant responses has been so great that reliable interpretation of its hormonal role has been difficult. The advent of gas–solid chromatography (see Chapter 2) has revolutionized the study of the role of ethylene in plants and, as a result, we are in the middle of an explosion of new interest in ethylene as a plant hormone.

2 Methods

All the books which deal with plant growth substances contain a certain amount of detailed information about the methods which are used in their study (e.g. PHILLIPS, 1971; AUDUS, 1973; LEOPOLD and KRIEDMANN, 1975; WAREING and PHILLIPS, 1978). Many of these methods are self-explanatory in their contexts, but it is essential here to look briefly at some of the experimental techniques which are in common use. Much of the work which has been done involves either studies on the responses of plants to applied plant growth substances or the separation, purification and assay of these substances from tissues. It is these last three aspects which are dealt with in this chapter.

One of the basic problems of the study of endogenous plant growth substances is the very small quantities which are present. The unit in which quantities of plant hormones are expressed is very often the microgram (μg), i.e. millionth of a gram, and extracts often contain only small fractions of a microgram. Concentrations of hormone solutions are normally expressed either in terms of molarity or in parts per million (ppm = mg dm^{-3} = μg cm^{-3}). In terms of normal chemistry these quantities are extremely minute and are very difficult to envisage. Most standard chemical techniques are far too insensitive to detect and measure quantities of substances of this order, so biological tests have to be employed once the growth substances have been extracted from the tissues containing them.

The methods used to extract and purify growth substances from plant tissues are very varied, and have tended to be developed on a rather *ad hoc* basis. A difficulty in selecting appropriate extraction procedures is that the substance being extracted may be of unknown chemical composition. It is thus impossible to be certain that the extraction procedures in use are not altering the structure and properties of the material. Some indole compounds are rather labile, especially under acid conditions, and serious losses can occur in the course of extraction unless adequate precautions are taken. Some gibberellin-like substances have been shown to change their properties after extraction if left for some time. In a good many cases one has no alternative but to put up with these inconvenient possibilities until techniques become available to overcome them, but it is important to bear in mind that such problems do exist, otherwise results may be misinterpreted.

2.1 Extraction procedures

The aim of all extraction procedures is to separate the plant growth

substance from everything else with as little loss as possible. Auxins and gibberellins are usually extracted by either diffusion or solvent extraction techniques.

2.1.1 *Diffusion techniques*

In this method the organ from which the substance is to be extracted is excised and placed with the cut surface on agar jelly, which may be replaced at intervals. Any substance moving from the tissue is subsequently extracted from the agar by other means.

The advantage of this technique is that while many substances present in the tissues do not readily diffuse into agar in this way, under appropriate conditions auxins and gibberellins do, so that the method combines extraction with partial purification. Went, in his early work, used the technique to demonstrate the diffusibility of auxin by cutting up the agar after diffusion, and placing pieces asymmetrically on decapitated oat coleoptiles in the dark. The subsequent curvature of these coleoptiles gave a measure of the amount of auxin which had diffused into the agar (Fig. 2–1). A disadvantage of the diffusion method is that it is really only practicable for small quantities of tissue. A further problem is that there is

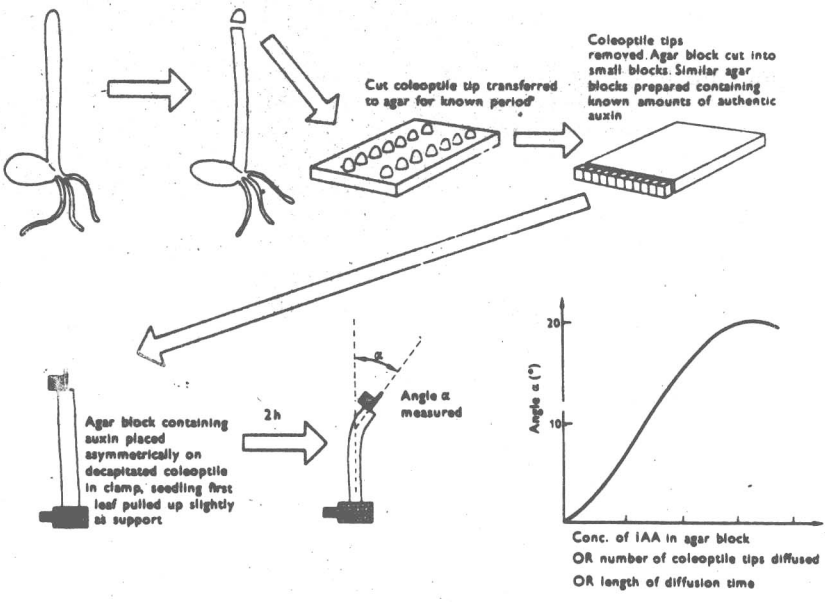

Fig. 2–1 An outline of the *Avena* curvature test for auxins, showing the relationship to Went's original quantitative studies referred to in Chapter 1. In the case illustrated the amount of auxin diffusing from the coleoptile tips could be estimated by reference to the standard curve prepared from the responses of coleoptiles to known concentrations of IAA.

no way of estimating the losses of activity which may take place due to chemical action at the cut surface. On the other hand, the technique has the advantage that it may be used to estimate the actual rate of production of substances by small pieces of tissue.

2.1.2 *Solvent extraction*

The principle employed here is that when a substance is shaken up with two immiscible solvents the proportion dissolved in each solvent will depend on the partition coefficient of the solute in the particular system. Since different substances have different partition coefficients, a series of transfers from one solvent to another can be used to 'leave behind' unwanted materials while purifying the required substance. At low pH

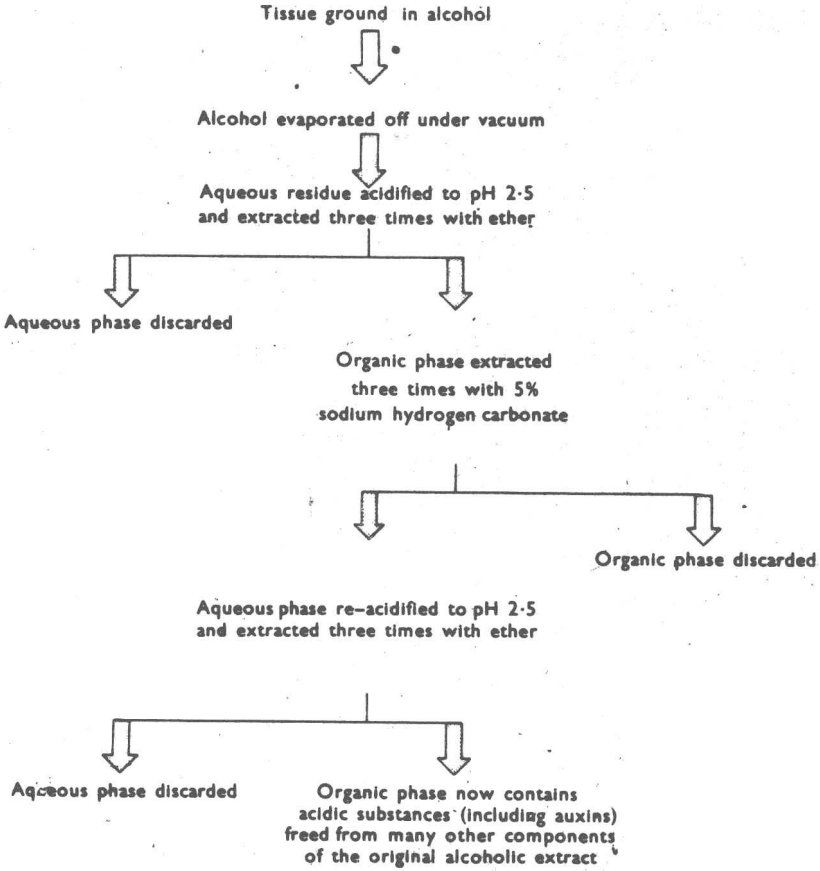

Fig. 2–2 Flow diagram illustrating an extraction procedure for acidic auxins in plant material.

values acidic substances are less ionized and are soluble in non-polar solvents, while at high pH, in their ionized form, they dissolve much more readily in polar solvents. The flow diagram in Fig. 2–2 illustrates typical procedure for the separation of an acidic substance from plant tissue.

The choice of solvents, pH values and other details depends on the substances being studied. In the case of gibberellins for example, ethyl acetate (ethyl ethanoate) is a much more satisfactory solvent than ethyl ether (ethoxyethane). By appropriate modification, solvent extraction techniques can also be used for substances which are neutral or basic rather than acidic.

Ethylene is unique amongst the plant growth substances in that it is a

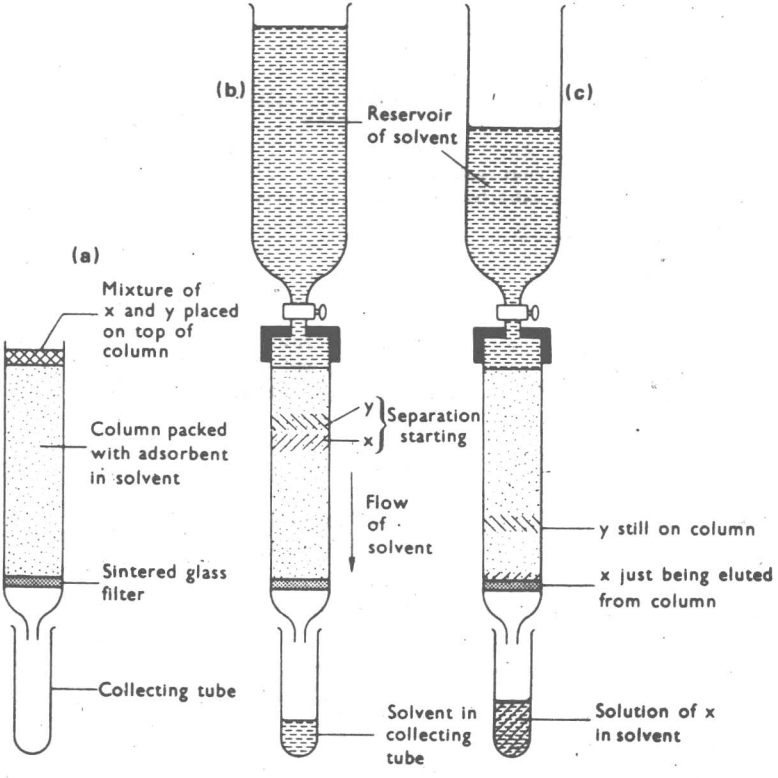

Fig. 2–3 Diagram illustrating the principle of column chromatography. (a) A mixture of two substances (x and y) is placed on top of the column. (b) The flow of solvent from the reservoir moves the substances down the column, x faster than y. (c) x is eluted from the column and collected while y is still on the way down.